APPENDIX: CLAIMS

What is claimed is:

1. A method of treating a solution of antibodies which may have virus activity, the method comprising

   a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and

   b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

2. The method of claim 1, wherein the anticomplement activity is reduced to less than about 60 CH_{50} units/mL.

3. The method of claim 1, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

4. The method of claim 3, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 30 CH_{50} units/mL.

5. The method of claim 1, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 60 CH_{50} units/mL.

6. The method of claim 5, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

7. The method of claim 1, wherein the incubation is for at least about ten days.

8. The method of claim 1, wherein the pH is maintained within a range of about 3.5 to about 5.0.

9. The method of claim 1, wherein the temperature is maintained within a range of 2° C to 50° C.

10. The method of claim 1, wherein the ionic strength is less than about 0.001 M.

11. The method of claim 1, wherein at least about 99% of the antibodies are monomeric.

12. The method of claim 1, comprising the further step of adjusting the tonicity of the solution to a physiologic value under such conditions that the ionic strength is not appreciably altered.

13. The method of claim 12, wherein the tonicity of the solution is adjusted by adding a carbohydrate to the solution.

14. The method of claim 13, wherein the carbohydrate used is maltose.

15. The method of claim 12, wherein the tonicity of the solution is adjusted to a range of about 230 to about 490 mosmol/kg solvent.

16. The method of claim 15, wherein the tonicity of the solution is adjusted to a range of about 274 to about 309 mosmol/kg solvent.

17. The method of claim 12, wherein the tonicity of the solution is adjusted by adding an amino acid to the solution.

18. The method of claim 17, wherein the amino acid used is glycine.

19. The method of claim 1, wherein the trialkylphosphate is tri-n-butyl phosphate and the detergent is selected from polysorbate 80 and sodium cholate.

20. The method of claim 1, wherein the solution has a pH between about 5.5 and about 6.0 during step a).

21. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001 M, a pH between about 3.5 and about 5.0, an antibody concentration of about 5% wt./wt., and a maltose concentration of about 10% wt./wt.

22. The preparation of claim 21, wherein the pH is about 4.25.

23. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.

24. The preparation of claim 23, wherein the pH is about 4.25.

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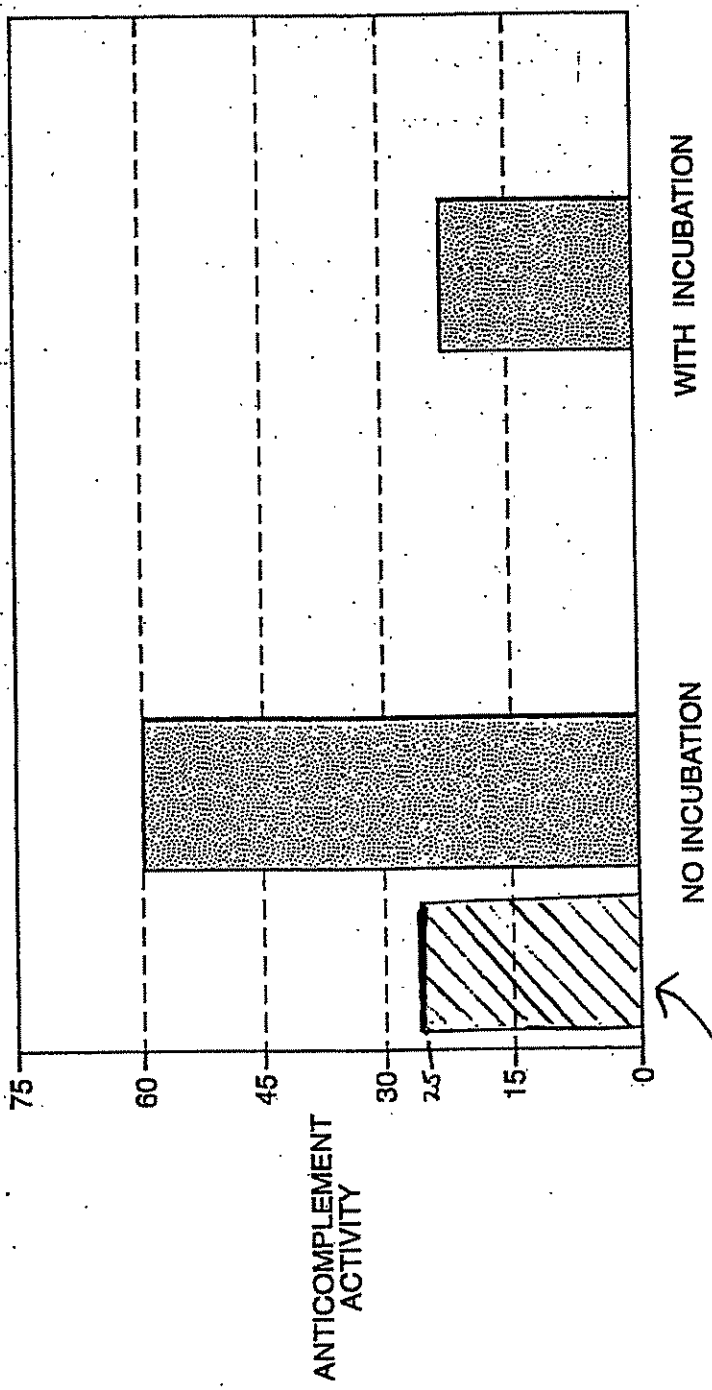
WITH INCUBATION
NO INCUBATION
75
60
45
30
25
15
0
ANTICOMPLEMENT ACTIVITY
Control (Tenold)
See TABLE 1, page 11.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/832,211	03/27/95	ALONSO	HSB 7222

18M2/1231

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EXAMINER	
EYLER, Y	
ART UNIT	PAPER NUMBER
1805	9

DATE MAILED: 12/31/97

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PAGE 105

PTO-90C (Rev. 2/95)

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BEFORE THE BOARD OF PATENT APPEALS

AND INTERFERENCES

Paper No. 9

Application Number: 08/532211

Filing Date: 9/22/95

Appellant(s): William R. Alonso

James A. Giblin

For Appellant

EXAMINER'S ANSWER

This is in response to appellant's brief on appeal filed 2/20/97.

(1) Real Party in Interest

A statement identifying the real party in interest is contained in the brief.

(2) Related Appeals and Interferences

A statement identifying the related appeals and interferences which will directly affect or be directly affected by or have a bearing on the decision in the pending appeal is contained in the brief.

(3) Status of Claims

The statement of the status of the claims contained in the brief is correct.

(4) Status of Amendments After Final

The appellant's statement of the status of amendments after final rejection contained in the brief is correct.

(5) Summary of Invention

The summary of invention contained in the brief is correct.

(6) Issues

The appellant's statement of the issues in the brief is substantially correct. The changes are as follows:

The rejection of claims 1, 3-6, 10, 21 and 23 under 35 U.S.C. 112 as vague and indefinite is withdrawn.

Claims 1-24 stand rejected under 35 U.S.C. 103 as unpatentable over Tenold (U.S. 4,396,608) in view of Neurath et al (U.S. 4,540,573), Mitra et al (U.S. 4,762,714) and Joy Yang et al. (Vox Sang. 67:337).

(7) Grouping of Claims

The appellant's statement in the brief that certain claims do not stand or fall together is not agreed with because claims 21-24 are dependent claims defining a product made by the process of claim 1. The product, as claimed, could not be infringed without also infringing the claimed method. Further, should the method of making the product be obvious, so too would the inherently resulting product made by using the method be obvious as well.

(8) Claims Appealed

The copy of the appealed claims contained in the Appendix to the brief is correct.

(9) Prior Art of Record

The following is a listing of the prior art of record relied upon in the rejection of claims under appeal. The following is a listing of the prior art of record relied upon in the rejection of claims under appeal.

4396608	Tenold	8/2/93
4540573	Neurath et al	9/10/85

4762714	Mitra et al	8/9/88

Joy Yang, Y.H. et al. "Antibody Fc functional activity of intravenous immunoglobulin preparations treated with solvent-detergent for virus inactivation" Vox Sang, Vol. 67, (May 17, 1994) pp. 337-334.

(10) New Prior Art

No new prior art has been applied in this examiner's answer.

(11) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

The rejection of Claims 1, 3-6, 10, 21 and 23 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention has been withdrawn.

Claims 1-24 are rejected under 35 U.S.C. § 103 as being unpatentable over Tenold (U.S. # 4,396,608) in view of Neurath et al (U.S.# 4,540,573), Mitra et al (U.S.#4,762,714), and Joy Yang et al (Vox Sang 67:337-344, 1994).

Tenold et al teach the modification of immune serum globulin (a solution of antibodies purified from blood plasma) to reduce anticomplement activity so that the solution may be safely administered intravenously. The starting material for Tenold's modifications is human immune serum globulin purified by Cohn's method, either fraction II or III. The starting solution is diluted in a physiologically acceptable carrier so as to obtain a protein or antibody

concentration of about 0.5-20%. The pH of the solution is than adjusted, and maintained, at about 3.5 to 5.0 with a physiologically acceptable acid such as hydrochloric acid. The temperature range is maintained at about 0-20 degrees C. The ionic strength of the solution is adjusted to less than 0.001 and the tonicity is adjusted, without altering the ionic strength, by addition of an amino acid such as glycine or a carbohydrate such as maltose. The specific osmolality of the final isotonic solution is not specified but the acceptable range to maintain tonicity would be well known and conventional to one of ordinary skill in the art. The final product obtained by the method of Tenold is an immune serum globulin, maintained at a controlled pH, temperature, ionic strength and tonicity so as to generate a monomeric solution of antibodies with a reduced anticomplement activity rendering the solution safe for intravenous administration. While Tenold does not incubate the solution at the given pH and temperature prior to the adjustment of ionic strength and tonicity, the pH and temperature are maintained throughout the procedure and the antibody solution is stored for up to six months under the defined controlled parameters. (see column 4 line 24 to column 8, line 54) The measured anticomplement activity of the immune serum globulin produced by Tenold's method is 3 mg protein per CH50 unit, which is less than 30,45 and 60 units. Tenold differs from the instant invention in that the starting material is not pre-treated to inactivate any infectious agents which may be present.

Neurath et al teach a method for the inactivation of infectious virus present in blood or blood derived solutions while maintaining the activity of proteins contained in the composition.

The method comprises treating the solution with a trialkylphosphate, for example tri-n-butyl phosphate and a wetting agent such as a detergent for example polysorbate 80 or sodium deoxycholate followed by removal of the inactivating agents and optional further processing of the product. The starting material for the method of Neurath et al may include fraction II or III of the Cohn purification to obtain immune serum globulin which is virus free (see the abstract, column 1 lines 5-20, column 4 lines 50-60, column 6 lines 40-61, column 7 to 8 and column 9 lines 19-25)

Mitra et al discuss the need to produce virus-free immune serum globulin solutions to assure that active viruses are not transmitted to patients. They follow the inactivation of spiked fraction II and III samples as the immune serum globulin solution is purified. They further specify that in order to obtain an immune serum globulin which is safe and effective for IV administration, the anticomplement activity of the solution must be reduced. They further specify that this may be accomplished through careful pH and ionic strength control (see column 1).

Joy Yang et al disclose a third-generation immune serum globulin for IV administration which includes a deliberate virus inactivation step of treatment with a solvent/detergent, specifically tri-n-butyl phosphate/polysorbate 80) (see the abstract and p.338, column 1). Joy Yang et al further discuss the desirability of retaining full Fc functions in a immune serum globulin preparation, including complement activity. Various assays are presented which evaluate the retention of Fc functions following the solvent/detergent treatment. An hemolysis

test indicates that, in their system, the complement mediated lysis of erythrocytes is not affected by the solvent/detergent virus inactivation step, see p. 339, column 2). Joy Yang et al do not teach the further adjustment of the immune serum globulin product to reduce anticomplement activity, but do stress the importance of full complement activity to the effectiveness of an immune serum globulin solution.

It would have been *prmia facie* obvious to and one of ordinary skill in the art would have been motivated, at the time the invention was made, to modify the method and resultant immune serum globulin product of Tenold et al by pretreatment of the antibody solution with a solvent/detergent as taught by Neurath et al or Joy Yang et al to ensure inactivation of infectious virus which is taught to be desirable by Mitra et al and to maintain a low anticomplement activity which is taught to be desirable by Mitra et al and Joy Yang et al.

(12) New Ground of Rejection

This examiner's answer does not contain any new ground of rejection.

(13) Response to argument

REJECTIONS UNDER 35 U.S.C. 112:

Appellant's arguments with regard to the rejection of claims 1, 3-6, 10, 21 and 23 are rendered moot by the withdrawal of the grounds of rejection.

REJECTIONS UNDER 35 U.S.C. 103:

Appellant's argue that there is no suggestion or motivation in the art to combine the teachings of Neurath et al. with the teachings of Tenold because the instantly observed increase

in ACA levels was unexpected and not taught in the prior art. Appellant's further argue, that even if the increase in ACA levels were expected, there was no suggestion in the art teaching the solution to the problem.

Appellant's arguments have been considered but are not found to be persuasive. While none of the prior art teaches an increase in ACA activity after viral inactivation by treatment with trialkylphosphate and detergent, the prior art in all cases indicates that it was art-standard knowledge that the level of ACA activity must be low for the serum globulin to be injected I.V. Tenold teach the undesirability of high levels of ACA activity and the necessity to assay for ACA activity and lower ACA activity before administration. (See column 1, lines 15-22 and column 2, lines 21-32). The method disclosed by Tenold et al to lower ACA activity is of record and is set forth supra. Mitra et al teach the desirability to prepare virus-free immune globulin compositions and further teach that ACA activity in such compositions was undesirable and must be eliminated, thus suggesting the combination of anti-viral treatment and the reduction of ACA levels. (See especially column 1, lines 23-45). Joy Yang et al in addition to teaching the desirability of viral inactivation of immune globulin compositions teach "it is clear the retention of the full range of Fc function is a prime requirement for therapeutic applications of IGIV.", i.e. ACA activity must be low. (See page 337, column 2, lines 1-3). Thus, the need to virally inactivate immune globulin compositions and to obtain immune compositions with low levels of ACA activity was known at the time the invention was made. Irregardless of the cause of the increased ACA level, it was art standard to measure

and lower levels of ACA in immune globulin preparations. The desirability of virus-free immune globulin compositions (blood products) was recognized. Neurath et al teaches a method of virally inactivating blood products. It would have been *prima facie* obvious and one of ordinary skill would have been motivated to treat immune globulin (solutions of antibodies) to inactivate viruses as taught by Neurath et al. Further, irregardless of whether it was expected that the anti-viral treatment of Neurath et al would result in increased ACA levels, the undesirability of high ACA levels was known and the need to measure ACA levels and lower them was known, thus providing motivation to combine the teachings of Tenold and Neurath, as taught by Tenold, Mitra et al, and Joy Yang et al. The reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. *In re Dillon*, 16 USPQ2d 1897 (Fed. Cir. 1990) and MPEP 2144.

Therefore, it is maintained that It would have been *prmia facie* obvious to and one of ordinary skill in the art would have been motivated, at the time the invention was made, to modify the method and resultant immune serum globulin product of Tenold et al by pretreatment of the antibody solution with a solvent/detergent as taught by Neurath et al or Joy Yang et al to ensure inactivation of infectious virus which is taught to be desirable by Mitra et al and to maintain a low anticomplement activity which is taught to be desirable by Mitra et al and Joy Yang et al.

For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

Yvonne Eyler, Ph.D.
Patent Examiner
Group 1800

December 23, 1997

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Patent
MSB-7232

P#10

I, Alishia Rowe-Babb, do hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor(s): W.D. Alonso

Application No: 08/532,211

Filed: September 22, 1995

Title: Preparation of Virally Inactivated IGIV.

Associate Power of Attorney and Change of Address

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The undersigned Attorney of Record hereby appoints Mary Boguslaski, Reg No. 31,486 as Associate Attorney for the above-entitled patent.

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Paper No. 13

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Ex parte WILLIAM R. ALONSO

Appeal No. 2001-1485
Application No. 08/532,211

HEARD: MAY 22, 2003[1]

Before WILLIAM F. SMITH, ADAMS and MOORE, *Administrative Patent Judges*.

MOORE, *Administrative Patent Judge*.

DECISION ON APPEAL

This is an appeal under 35 U.S.C. § 134 from the final rejection of claims 1 - 24, which are all of the claims pending in this application.

1 A request for oral hearing was made within the notice of appeal dated December 18, 1996. Although the appellant appears to have been charged the appeal and oral hearing fees on January 16, 1997, the request for oral hearing was not acted upon by the USPTO. As a historical note, we observe that at the time the request was filed, 37 CFR 1.194 (b) (1993) read "If appellant desires an oral hearing, appellant must file a written request . . ." 37 CFR 1.194 (b) (1997) now reads "If appellant desires an oral hearing, appellant must file, in a separate paper, a written request for such hearing . . ." Such oversights are now more easily avoided. We sincerely apologize for the delay in discovering the oral hearing request.

REPRESENTATIVE CLAIM

Claim 1 is representative of the claims on appeal and reads as follows:

1. A method of treating a solution of antibodies which may have virus activity, the method comprising:

a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and

b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

The References

In rejecting the claims under 35 U.S.C. § 103(a) the examiner relies upon the following references:

Tenold (Tenold)	4,396,608	Aug. 02, 1983
Neurath et al. (Neurath)	4,540,573	Sep. 10, 1985
Mitra et al. (Mitra)	4,762,714	Aug. 09, 1988

Joy Yang, Y.H. et al., "Antibody Fc Functioning Activity of Intravenous Immunoglobulin Preparations Treated with Solvent-Detergent for Virus Inactivation," Vox Sang, 1994; 67:337-344 (Joy Yang).

The Rejections

Claims 1-24 stand rejected under 35 U.S.C.§ 103(a) as being unpatentable over Tenold in view of Neurath, Mitra, and Joy Yang.

The Invention

The invention is directed to a method for treating a solution of antibodies which may have viral activity by a two-step process of first contacting the solution with a trialkylphosphate and detergent under conditions which reduce viral activity and increase anticomplement activity, then incubating the solution under controlled time, pH, temperature, and ionic strength to reduce the increased anticomplement activity. (Claim 1).

Discussion

The § 103 Rejection of Claims 1-24 over Tenold in view of Neurath, Mitra, and Joy Yang

The examiner has found that Tenold teaches the modification of immune serum globulin (ISG) to reduce anticomplement activity (ACA) in order that the serum may be administered safely. (Examiner's Answer, page 4, lines 15-17). The resulting ISG product is then maintained at a controlled pH, temperature, ionic strength, and tonicity so as to generate a monomeric solution of antibodies with a reduced ACA rendering the solution safe for intravenous administration (Id., page 5, lines 7-11).

The examiner has also found that Neurath discloses a method for inactivating infectious virus present in blood or blood derived solutions (including ISG) while maintaining the activity of proteins contained in the composition. This is accomplished by treating the solution with a trialkylphosphate and a detergent

followed by removal of the inactivating agents and further optional processing of the product. (Id., page 5, line 19 - page 6, line 7).

The examiner has additionally found that Mitra teaches the need to produce virus-free ISG to prevent viral infection in patients. Mitra also recognizes the historic need to reduce the ACA to obtain safe ISG. (Id., page 6, lines 8-14).

The examiner has further found that Joy Yang discloses an ISG with a deliberate virus inactivation step followed by retention of complement activity. (Id., page 6, line 15 - page 7, line 8).

The examiner thus concludes that it would have been obvious to one of ordinary skill at the time the invention was made to modify Tenold to pretreat for viral reduction as taught by Neurath, Mitra, and Joy Yang to both ensure reduction of viruses and low ACA. (Id., page 7, lines 6-11). As to the incubation step of Claim 1(b), the examiner explains that the ``antibody solution [of Tenold] is stored for up to six months under the defined controlled parameters,'' citing Tenold, column 4, line 24 - column 8, line 54).

The appellant, on the other hand, asserts that there is no suggestion or motivation to require a step (b) which reduces the increased ACA level as no one was aware of the ``surprising'' increase. Consequently, no one could have expected the increased ACA level, much less found a way to counter it. (Appeal Brief, page 4, lines 4-34).

The appellant also asserts that the Tenold and Mitra references do not teach a decrease in ACA, and Tenold blames the increase of ACA on aggregation of the monomers. (Id., page 5, lines 1-11). Mitra, it is urged, fails to disclose a lowering of ACA due to incubation conditions. (Id, page 5, lines 12-18).

We observe that it is not in dispute that the appellant's process combines two relatively well-known steps to accomplish known functions. Neurath is known to provide acceptable viral inactivation (Neurath, column 4, lines 1-18), and Tenold to provide ISG solutions with low ACA (Tenold, column 8, lines 8-10). Indeed, that is the basis for the examiner's rejection - inactivation of viruses and a low ACA are required for intravenous preparations - therefore it would have been obvious to pretreat the Tenold starting material to eliminate viruses. (Examiner's Answer, page 9, lines 1-20).

The examiner notes that none of the applied prior art teaches an increase in ACA activity after viral inactivation by treatment with trialkylphosphate and detergent, but also asserts that it was art-standard knowledge that the level of ACA must be low for the serum globulin to be injected intravenously (Examiner's Answer, page 8, lines 4-8).

However, the claimed subject matter requires that the inactivation step result in an increase in ACA levels, and a reduction in that claimed increase by the incubation step to a point where the solution is suitable for intravenous use. The

appellant argues that there is no motivation to require an incubation step (b) as the increase in ACA caused by using the solvent-detergent method was unexpected. (Appeal Brief, page 4, lines 8-10). The examiner has admitted that the prior art is silent on this claimed increase in ACA.

It is clear to us that the problems of viral presence in antibody solutions and the problems of reducing ACA to an acceptable level were well known, as discussed in the cited references. The solvent-detergent method of Neurath inactivates viruses, and the Tenold ACA reduction process reduces ACA. The appellant has admitted that the combination of the Neurath and Tenold procedures ``may have been an obvious step'' (Appeal Brief, page 4, lines 4-5) but that such combination ``would only result in step (a)'' (Id., page 4, lines 6-7).

The appellant has discovered that Neurath's process results in elevated ACA levels (Specification, page 17, last 2 lines). Although the ACA increase was unrecognized, Neurath alone therefore inherently meets step (a) of the process. Neurath also suggests ``further processing'' (column 9, lines 19-24). The question then presented is whether one of ordinary skill in the art would be taught to follow with the Tenold process and whether the instantly claimed results would be obtained.

Tenold discloses a method for reducing ACA in ISG to the point that the ISG is suitable for IV administration. This is accomplished by solubilizing an ISG to yield a solution with a

certain protein concentration. The pH and ionic strength of the solution is adjusted to the point where the monomer content of the ISG is greater than about 90% and the actual and latent ACA is such that the ISG product is IV injectable. (Tenold, column 4, lines 30-41). The examiner states that Tenold differs from the instant claims in that the starting material is not pre-treated to inactivate infectious agents (Examiner's Answer, page 5, lines 16-18). The appellant urges that Tenold already has a low ACA and consequently cannot reduce ACA. (Appeal Brief, page 5, lines 3-6).

Tenold also discloses storing the solutions at an ionic strength of 0.001, a pH of 4.2, at room temperature, and for a six-month period of time. (Tenold, column 9, lines 12-21). The specification reveals that the incubation is conducted at an ionic strength of 0.001, a pH of 4.25, at 20-27°C (room temperature), at not less than 21 days (Specification, page 9, lines 4-12). Thus, Tenold would appear to disclose the values required by step (b) to obtain the desired ACA goal.

Viewed alone, the relied upon teachings of the applied prior art may perhaps be said to support a conclusion of prima facie obviousness.

However, the specification establishes the following:

(1) Solvent detergent viral inactivation results in an increase in ACA (See Table 1, Specification, Page 11).

(2) Using the solvent detergent process to treat ISG and subsequently treating that product according to Tenold does not

result in a product having acceptable ACA levels when measured immediately. (Specification, paragraph bridging pages 2 and 3 and Table 5).

(3) In contrast, holding (``incubating'') the solvent-detergent inactivated samples results in marked lowering of ACA (Specification, page 12, Table 3).

(4) The ACA results do not appear to correlate to the monomer content (Specification, page 17, table 8).

(5) Tenold's basic process (starting with non-solvent detergent inactivated solutions) results in a 25 ACA (CH_{50}/mL). (Specification, page 11, table 1).

From this, it is apparent that the problem being addressed places the question of whether a prima facie case of obviousness exists in a different light. First, one must question whether the teachings and results of Tenold can be combined with Neurath successfully. See, for example, the paragraph bridging pages 2 and 3 of the specification. Tenold starts with an unmodified human ISG (Tenold, column 4, lines 65-66) initially having an ACA which is unacceptable for intravenous injection (although the actual ACA level is not specifically described) (Tenold, Column 1, lines 23-27). The ACA level is lowered such that the final product has an ACA which is acceptable immediately, without appreciable change in the monomer content after 6 months (Column 8, lines 8-10). From the evidence provided by the appellants, this ACA can initially be 25 (Specification, page 11, Table 1).

If one of skill in the art starts with the Neurath solvent detergent modified ISG, and further treats that product by the Tenold process, the ISG would apparently still have an unacceptable ACA level. (See specification, table 3, page 12).

The examiner does not dispute the data in the specification showing that simply treating a solvent detergent virally inactivated ISG solution obtained by way of the Neurath process will not have an acceptable ACA level immediately or shortly after being further treated by the procedure described in Tenold. Rather, the examiner relies upon the data reported after containers of the Tenold treated ISG had been stored for six months.

Specifically, Tenold states at column 9, lines 15-30 that initial results indicated that a monomer level of 99% had been achieved. That level of monomer content had been maintained for six months. How does the Tenold data compare with the data in the present specification? Not well.

The appellants state that they treated solvent detergent virally inactivated ISG obtained by way of the Neurath process with the Tenold ACA lowering procedure and that the resulting product did not have an initially acceptable ACA level. This is in direct contrast to Tenold's statements that the process initially provides an acceptable ACA. Confronted with this anomaly, why would one of ordinary skill in the art then further incubate the solvent detergent treated ISG having an unacceptable

ACA after the Tenold process?[2] On this record we find no reason to do so.

The six-month data in Tenold only shows that an initial acceptable ACA level can be maintained upon six months storage. Importantly, Tenold does not teach that the initially high ACA level may be lowered merely by storing the ISG for six months. Assuming the examiner is correct, and that one of skill in the art would measure ACA after Neurath's solvent detergent treatment, that person would presumably discover what the appellants did; the ISG has a higher ACA level than expected. Why, then would one skilled in the art know that simply treating the solvent detergent ISG by way of Tenold would not lower the ACA to an acceptable level, but rather a significant incubation step would be needed? Again Tenold only indicates that six months storage maintains, not lowers, the ACA level.

> ``Obviousness does not require absolute predictability of success. Indeed, for many inventions that seem quite obvious, there is no absolute predictability of success until the invention is reduced to practice. There is always at least a possibility of unexpected results, that would then provide an objective basis for showing that the invention, although apparently obvious, was in law nonobvious.'' In re O'Farrell, 853 F.2d 894, 903, 7 USPQ2d 1673, 1681 (Fed. Cir. 1988).

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2 Although not discussed in the Examiner's Answer or the Brief, we observe that Mitra teaches a Cohn fractionated ISG, when stored, shows a reduction in the AIDS virus. (Column 6, lines 42-54 and column 7, line 1 to column 8, line 25). However, this storage does not occur after a solvent detergent inactivation step, and does not reveal the effect on the ACA of the ISG solution.

We think this is the case here. Once appellants did what the prior art would reasonably appear to suggest doing, they found they did not obtain the expected results. It was only after obtaining the anomalous results did they understand the problem and discover its solution.

The decision of the examiner is reversed.

Summary of Decision

The rejection of claims 1-24 under 35 U.S.C. §103(a) as being unpatentable over Tenold in view of Neurath, Mitra, and Joy Yang is reversed.

REVERSED

WILLIAM F. SMITH
Administrative Patent Judge

DONALD E. ADAMS
Administrative Patent Judge

JAMES T. MOORE
Administrative Patent Judge

BOARD OF PATENT APPEALS AND INTERFERENCES

Appeal No. 2001-1485
Application No. 08/532,211

CONNOLLY BOVE LODGE & HUTZ, LLP
1220 N. MARKET STREET
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JM/ki

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NOTICE OF ALLOWANCE AND FEE(S) DUE

23416 7590 09/11/2003

CONNOLLY BOVE LODGE & HUTZ, LLP
P O BOX 2207
WILMINGTON, DE 19899

EXAMINER
EYLER, YVONNE L

ART UNIT	CLASS-SUBCLASS
1646	435-236000

DATE MAILED: 09/11/2003

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/532,211	09/22/1995	WILLIAM R. ALONSO	MSB-7232	7468

TITLE OF INVENTION: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1300	$0	$1300	12/11/2003

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE REFLECTS A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE APPLIED IN THIS APPLICATION. THE PTOL-85B (OR AN EQUIVALENT) MUST BE RETURNED WITHIN THIS PERIOD EVEN IF NO FEE IS DUE OR THE APPLICATION WILL BE REGARDED AS ABANDONED.

HOW TO REPLY TO THIS NOTICE:

I. Review the SMALL ENTITY status shown above.

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B. If the status is changed, pay the PUBLICATION FEE (if required) and twice the amount of the ISSUE FEE shown above and notify the United States Patent and Trademark Office of the change in status, or

If the SMALL ENTITY is shown as NO:

A. Pay TOTAL FEE(S) DUE shown above, or

B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check the box below and enclose the PUBLICATION FEE and 1/2 the ISSUE FEE shown above.

☐ Applicant claims SMALL ENTITY status. See 37 CFR 1.27.

II. PART B - FEE(S) TRANSMITTAL should be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). Even if the fee(s) have already been paid, Part B - Fee(s) Transmittal should be completed and returned. If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PTOL-85 (Rev. 08/03) Approved for use through 04/30/2004.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: Mail Mail Stop ISSUE FEE
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CURRENT CORRESPONDENCE ADDRESS (Note: Legibly mark-up with any corrections or use Block 1)

23416 7590 09/11/2003

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Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/532,211	09/22/1995	WILLIAM R. ALONSO	MSB-7232	7468

TITLE OF INVENTION: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1300	$0	$1300	12/11/2003

EXAMINER	ART UNIT	CLASS-SUBCLASS
EYLER, YVONNE L	1646	435-236000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.

☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

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3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the USPTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ individual ☐ corporation or other private group entity ☐ government

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☐ The Director is hereby authorized by charge the required fee(s), or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

Director for Patents is requested to apply the Issue Fee and Publication Fee (if any) or to re-apply any previously paid issue fee to the application identified above.

(Authorized Signature) (Date)

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This collection of information is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, Alexandria, Virginia 22313-1450.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/532,211	09/22/1995	WILLIAM R. ALONSO	MSB-7232	7468

EXAMINER	
EYLER, YVONNE L	

ART UNIT	PAPER NUMBER
1646	

23416 7590 09/11/2003

CONNOLLY BOVE LODGE & HUTZ, LLP
P O BOX 2207
WILMINGTON, DE 19899

DATE MAILED: 09/11/2003

Determination of Patent Term Extension under 35 U.S.C. 154 (b)
(application filed after June 7, 1995 but prior to May 29, 2000)

The Patent Term Extension is 1772 day(s). Any patent to issue from the above-identified application will include an indication of the 1772 day extension on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Extension is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) system (http://pair.uspto.gov).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (703) 305-1383. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 08/03) Approved for use through 04/30/2004.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/532,211	09/22/1995	WILLIAM R. ALONSO	MSB-7232	7468

EXAMINER	
EYLER, YVONNE L	

ART UNIT	PAPER NUMBER
1646	

23416 7590 09/11/2003

CONNOLLY BOVE LODGE & HUTZ, LLP
P O BOX 2207
WILMINGTON, DE 19899

DATE MAILED: 09/11/2003

Notice of Fee Increase on October 1, 2003

If a reply to a "Notice of Allowance and Fee(s) Due" is filed in the Office on or after October 1, 2003, then the amount due will be higher than that set forth in the "Notice of Allowance and Fee(s) Due" since there will be an increase in fees effective on October 1, 2003. See Revision of Patent Fees for Fiscal Year 2004; Final Rule, 68 Fed. Reg. 41532, 41533, 41534 (July 14, 2003).

The current fee schedule is accessible from (http://www.uspto.gov/main/howtofees.htm).

If the fee paid is the amount shown on the "Notice of Allowance and Fee(s) Due" but not the correct amount in view of the fee increase, a "Notice of Pay Balance of Issue Fee" will be mailed to applicant. In order to avoid processing delays associated with mailing of a "Notice of Pay Balance of Issue Fee," if the response to the Notice of Allowance is to be filed on or after October 1, 2003 (or mailed with a certificate of mailing on or after October 1, 2003), the issue fee paid should be the fee that is required at the time the fee is paid. If the issue fee was previously paid, and the response to the "Notice of Allowance and Fee(s) Due" includes a request to apply a previously-paid issue fee to the issue fee now due, then the difference between the issue fee amount at the time the response is filed and the previously-paid issue fee should be paid. See Manual of Patent Examining Procedure, Section 1308.01 (Eighth Edition, August 2001).

Effective October 1, 2003, 37 CFR 1.18 is amended by revising paragraphs (a) through (c) to read as set forth below.

Section 1.18 Patent post allowance (including issue) fees.

(a) Issue fee for issuing each original or reissue patent, except a design or plant patent:
By a small entity (Sec. 1.27(a)) $665.00
By other than a small entity $1,330.00

(b) Issue fee for issuing a design patent:
By a small entity (Sec. 1.27(a)) $240.00
By other than a small entity $480.00

(c) Issue fee for issuing a plant patent:
By a small entity (Sec. 1.27(a)) $320.00
By other than a small entity $640.00

Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at (703) 305-8283.

PTOL-85 (Rev. 08/03) Approved for use through 04/30/2004.

Notice of Allowability	Application No. 08/532,211	Applicant(s) ALONSO, WILLIAM R.	
	Examiner eyler yvonne	Art Unit 1646	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the board decision of 7/29/03*.
2. ☒ The allowed claim(s) is/are *1-24*.
3. ☐ The drawings filed on _____ are accepted by the Examiner.
4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____.
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.
5. ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).
   (a) ☐ The translation of the foreign language provisional application has been received.
6. ☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application. **THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

7. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

8. ☒ CORRECTED DRAWINGS must be submitted.
   (a) ☒ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No. *2*.
   (b) ☒ including changes required by the proposed drawing correction filed *5/14/96*, which has been approved by the Examiner.
   (c) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No. _____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet.

9. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1☐ Notice of References Cited (PTO-892)
2☐ Notice of Informal Patent Application (PTO-152)
3☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
4☐ Interview Summary (PTO-413), Paper No._____.
5☐ Information Disclosure Statements (PTO-1449), Paper No. _____.
6☐ Examiner's Amendment/Comment
7☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
8☐ Examiner's Statement of Reasons for Allowance
9☐ Other

YVONNE EYLER, PH.D
SUPERVISORY PATENT EXAMINER
TECHNOLOGY CENTER 1600

MSB-7232 (7430-190)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

WILLIAM R. ALONSO	:	
SERIAL NO.: 08/532,211	:	ART UNIT: 1646
FILING DATE: SEPTEMBER 22, 1995	:	EXAMINER: Eyler, Yvonne L.
FOR: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN	:	

M.S. Issue Fee
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I hereby certify that this correspondence is being deposited with the U.S. Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on this 22nd day of October 2003.
BY: Jean M. Marshall
Jean M. Marshall

LETTER TO OFFICIAL DRAFTSPERSON

Please enter the enclosed (1) sheet of drawings as the formal drawing in this case, in compliance with changes required by the Notice of Allowability of September 11, 2003 and proposed drawing correction submitted by Applicant on May 14, 1996. A copy of the Notice of Draftsperson's Drawing Review, PTO-948, of November 15, 1995, is also attached, showing that no changes were required.

If there are any additional fees due in connection with the filing of this response, including any fees required for an additional extension of time under 37 C.F.R §1.136, such an extension is requested and the Commissioner is authorized to charge or credit any overpayment to Deposit Account No. 03-2775.

Respectfully submitted,
CONNOLLY BOVE LODGE & HUTZ LLP

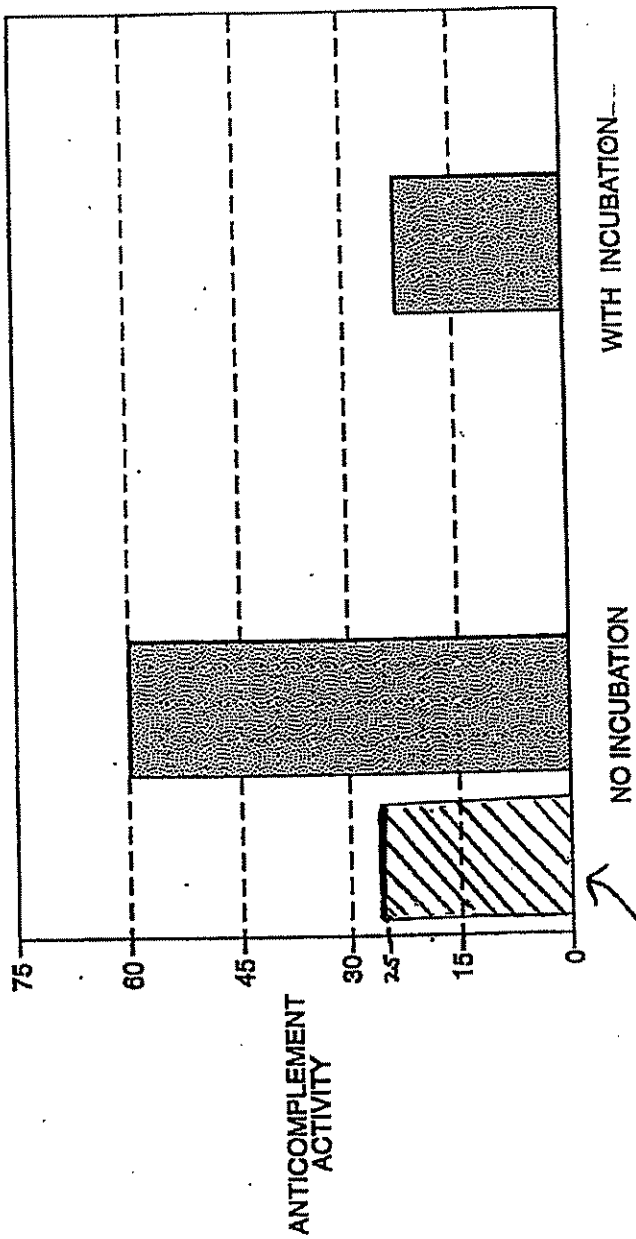
By Christine M. Hansen
Christine M. Hansen
Reg. No. 40,634
Tel. (302) 658-9141

:ODMA\MHODMA\CB;294864;1

08/533,211

MSB-7232

1/1



Control (Tenoted)
See Table 1, page 11.

08/532,211

MSB-7232 Replacement Sheet

1/1

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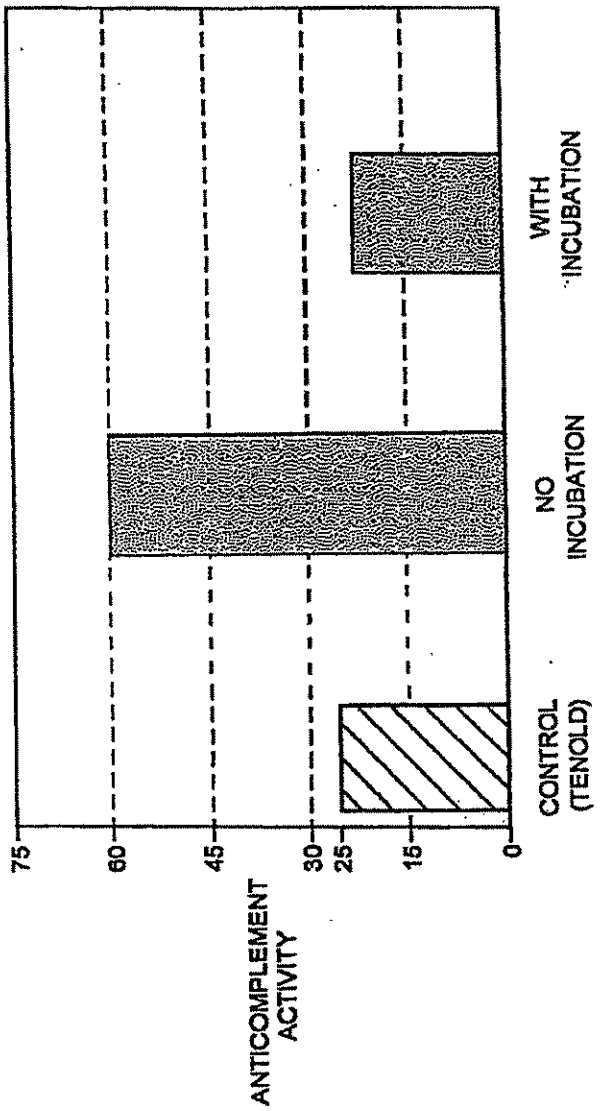

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Jean M. Marshall	(Signature)
December 10, 2003	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
08/532,211	09/22/1995	WILLIAM R. ALONSO	MSB-7232	7468

TITLE OF INVENTION: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN .

APPLN. TYPE	SMALL ENTITY	ISSUE FEE	PUBLICATION FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	$1300	$0	$1300	12/11/2003

EXAMINER	ART UNIT	CLASS-SUBCLASS
EYLER, YVONNE L	1646	435-236000

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COMMUNICATION ACCOMPANYING
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WILLIAM R. ALONSO

SERIAL NO. 08/532,211

FILED: SEPTEMBER 22, 1995

FOR: PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

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Respectfully submitted,
CONNOLLY BOVE LODGE AND HUTZ LLP

By Christine M. Hansen
Christine M. Hansen
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Encl. 1) Check
2) Issue Fee Transmittal

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(12) **United States Patent**
Alonso

(10) **Patent No.: US 6,686,191 B1**
(45) **Date of Patent: Feb. 3, 2004**

(54) **PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN**

(75) Inventor: **William R. Alonso, Cary, NC (US)**

(73) Assignee: **Bayer HealthCare LLC, Tarrytown, NY (US)**

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1772 days.

(21) Appl. No.: **08/532,211**

(22) Filed: **Sep. 22, 1995**

(51) Int. Cl.[7] C12N 7/04; A61K 39/395; A61K 39/40; A61K 39/42

(52) U.S. Cl. 435/236; 424/176.1; 424/177.1; 424/130.1

(58) Field of Search 530/390.1, 390.5, 530/386, 387.1; 424/176.1, 177.1, 130.1; 435/236

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,396,608 A * 8/1983 Tenold
4,540,573 A * 9/1985 Neurath et al.
4,762,714 A * 8/1988 Mitra et al.

OTHER PUBLICATIONS

Joy Yang, Y.H. et al. "Antibody Fc functional activity of intravenous immunoglobulin preparations treated with solvent–detergent for virus inactivation" Vox Sang, vol. 67, pp. 337–344, May 17, 1994).*

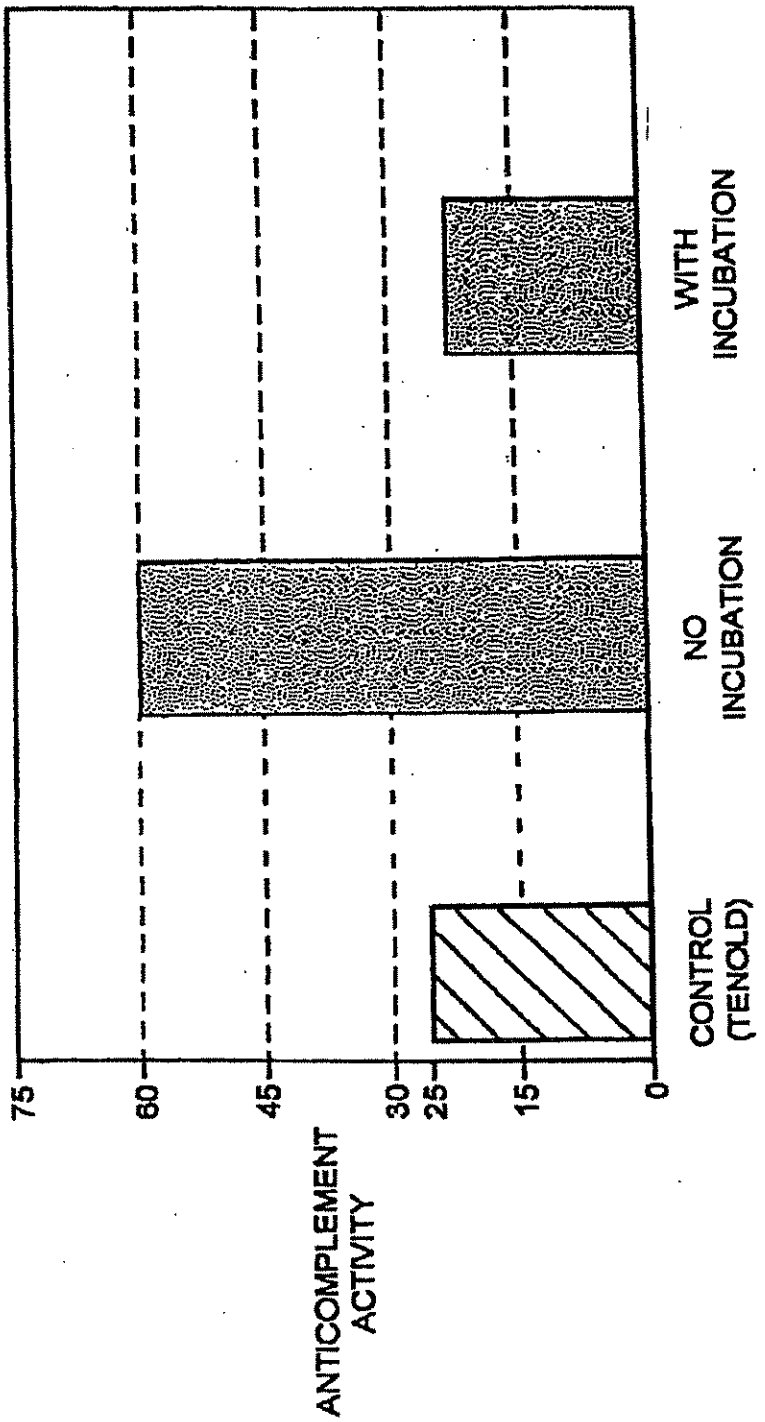
* cited by examiner

Primary Examiner—Yvonne Eyler
(74) *Attorney, Agent, or Firm*—Connolly Bove Lodge & Hutz LLP

(57) **ABSTRACT**

Method of reducing the anticomplement activity (ACA) resulting from viral inactivation treatment of a solution of antibodies, the method comprising contacting the solution with a trialkylphosphate, such as tri-n-butyl phosphate, and a detergent, such as sodium cholate, under conditions sufficient to reduce substantially the virus activity, and then incubating the solution under controlled conditions of time, pH, temperature, and ionic strength such that the anticomplement activity is reduced to an acceptable level. In a preferred embodiment, the ACA is reduced to less than 60 CH_{50} units/mL, the incubation is for at least about ten days at a pH from 3.5 to 5.0, the temperature is maintained within a range of 2 to 50° C., and the ionic strength of the solution is less than about 0.001 M.

24 Claims, 1 Drawing Sheet


WITH INCUBATION
NO INCUBATION
CONTROL (TENOLD)
75
60
45
30
25
15
0
ANTICOMPLEMENT ACTIVITY

PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

BACKGROUND OF THE INVENTION

1. Field

This invention generally deals with an intravenously injectable immunoglobulin product, and more specifically deals with an intravenously injectable immune serum globulin (IGIV) which has been subjected to a virus inactivation step and which has a low level of anticomplement activity.

2. Background

Early pharmaceutical preparations of immune serum globulins could not be administered intravenously due to an unacceptably high incidence of adverse reactions. These adverse reactions were associated with a decrease in serum complement levels, apparently caused by complement binding to the administered gamma globulin. (1) The ability of gamma globulin to bind complement, or its anticomplement activity (ACA), is greatly increased as a result of denaturation brought about during the fractionation procedure. Several approaches have been taken to address the problem of rendering ISG safe for intravenous administration. (See (2) and references therein). Tenold reported a method of preparing an immune serum globulin (ISG) with low ACA which could be administered by intravenous injection. (2, incorporated herein by reference). The Tenold '608 process requires formulating the ISG at low ionic strength (preferably less than about 0.001) and at low pH (3.5–5.0).

Other methods of preparing intravenously injectable immune serum globulin (IGIV) have been reported, including stabilizing with carbohydrates such as maltose (3). A process including incubation of ISG at pH 4.0 at 37° C. (4) results in a product with low ACA which may be administered by intravenous injection; however, upon storage the product regains its high ACA. IGIV has also been prepared by covalent modification of the ISG, for example by proteolysis (5) or by reduction of disulfide linkages followed by reaction with a blocking agent (1,6).

Antibody preparations, since they are isolated blood products, have an inherent hazard of transmitting virally-mediated diseases. Inactivation of viruses is an important step in producing safe and effective blood products. U.S. Pat. No. 4,540,573 to Neurath et al., which is incorporated herein by reference, describes a viral inactivation process using a trialkyl phosphate and detergent process (hereinafter, the solvent/detergent process, or SD process). (7) That solvent/detergent method has gained acceptance as being efficacious in the inactivation of lipid-enveloped viruses with limited adverse effects on biological activity or blood product profile. (8, 15; See also 12 for a discussion of various viral inactivation processes).

Current antibody preparations on the market generally have been regarded as safe with respect to viral contamination. (9) This is thought to be due to features of the fractionation processes used to isolate these blood products. However, it would be desirable to further ensure the safety of the antibody preparations by including a distinct viral inactivation step in the production process. Successful reduction of viral activity in an IGIV solution was reported using several different methods of viral inactivation for a variety of viruses. (16, 17) A process for preparation of immunoglobulins substantially free of retrovirus has been reported involving incubation of ISG under controlled conditions of time, temperature, and pH. The process entails

isolating ISG via a cold ethanol plasma fractionation process and then storage of the ISG at one of two storage conditions: (a) at pH≦4.25 at a temperature of 27° C. for at least three days, or (b) at pH≦6.8 at a temperature of 45° C. for at least six hours. (10).

We have found that using the SD process to treat ISG preparations, especially those subsequently formulated according to the Tenold '608 patent, results in a product with an acceptable viral inactivation but with unacceptably high levels of ACA. Elevated ACA levels were always detected at the sterile bulk stage (i.e., after compounding as 5% or 10% IGIV and filtration with 0.2 μm sterile filters) of all tri-n-butyl phosphate (TNBP)/detergent treated IGIV preparations regardless of process scale. Preparations of ISG with high ACA levels are not suitable for intravenous injection and instead must be administered via other routes, e.g. intramuscular (IM) injection. However, IGIV preparations are more desirable since they are immediately available in the bloodstream and are not subject to loss associated with IM injection. It is thus desirable to have an IGIV product which is both low in ACA and has been subjected to a viral inactivation step.

SUMMARY OF THE INVENTION

The invention is a method for producing an intravenously injectable immune serum globulin (IGIV) preparation with low anticomplement activity which has been chemically treated to render it substantially free of lipid-enveloped viruses. The method comprises a solvent/detergent viral inactivation step followed by an incubation step. We have discovered that the incubation step is necessary to achieve an acceptable level of ACA low enough to allow the ISG to be administered by intravenous injection. The incubation step should be conducted under controlled time, pH, temperature, and ionic strength. Preferably, the pH should be maintained between about 3.5 and about 5.0, the temperature should be within a range of about 2 to about 50° C., and the ionic strength should be less than about 0.001M. In a preferred embodiment the ACA of the ISG preparation decreases gradually over a period of at least about ten days when the ISG is maintained at a pH of about 4.25 at low ionic strength (less than about 0.001M) and the viral inactivation step (in a model system) results in a substantial reduction (i.e. at least 4 logs) in the titer of lipid enveloped viruses.

BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 shows a comparison of the typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation of the present invention.

SPECIFIC EMBODIMENTS

Materials and Methods

The starting material for the process of this invention is unmodified human immune serum globulin. In the specification and claims the term "immune serum globulin" is used to define the substance also referred to in the literature variously as gamma globulin, IgG and immunoglobulin G. It consists predominantly and preferably of at least about 85 percent of the 7S species of gamma globulin, which has a molecular weight of about 160,000. Any remainder is preferably 9S species, with a molecular weight of about 300,000. Both standard immune and hyperimmune serum globulins, e.g., tetanus, rabies and hepatitis immune serum globulins,

can be employed, the solvent/detergent treated product being immune and hyperimmune ISG, respectively. Thus, a suitable starting material for the process of this invention is Cohn's Fraction II or Fraction III filtrate. (See Refs. 13, 14.)

Fraction II, by ultracentrifugation studies, is predominantly (about 85 percent) the 7S (sedimentation constant of 7) species of gamma globulin with an average molecular weight of 160,000. The remaining protein is essentially 9S material with a M.W. of about 300,000. Wet Fraction II paste (approximately 30 percent solids) is commonly lyophilized to obtain dry ISG powder which is then dissolved and prepared for intramuscular injection as a 16.5 percent sterile solution. Either the wet Fraction II paste or the dry ISG powder is a suitable starting material for the process of this invention.

Gamma globulin obtained by any process which has essentially the same composition of protein components as found in the Cohn Fraction II or Fraction III filtrate can be used as starting material in the present process. Both standard immune serum globulin and hyperimmune serum globulin can be employed as starting materials. As is well known, the latter is produced from plasma or serum obtained from selected donors who have much higher titers for a specific antibody than is normally found in the average population. These donors have either been recently immunized with a particular vaccine or else they have recently recovered from an infection or disease. These high titer sera or plasmas are pooled and subjected to the usual Cohn fractionation procedures up to the point of isolating Fraction II.

Furthermore, because the amount of antibody required to achieve a desired immunological response is substantially less when administered intravenously, it will be apparent the intravenous dose will be substantially less than the intramuscular dose which will produce the same serum antibody titer. Thus, the dose of intramuscular ISG and hyperimmune serum globulin must be higher than that required to achieve the same serum antibody titer when globulin of the same antibody activity is administered intravenously.

The starting wet paste or lyophilized powder is dissolved in a volume of water or other physiologically-acceptable carrier to provide a protein solution of a concentration of about 0.5–20% preferably about 5 to 10 percent. If Fraction III filtrate is employed, the aqueous solution must be concentrated by conventional techniques to the desired protein concentration. Any protein concentration may be used in this method; however, the above range is preferred from a practical standpoint.

After the protein has been dissolved or concentrated, the solution is adjusted to a pH of about 3.5 to 5.0 preferably about 3.8 to 4.2, by addition of a physiologically-acceptable acid such as hydrochloric acid. In general, the pH is adjusted to a point whereat the monomeric material in the protein solution is maintained at a maximum. However, the pH must not be so low as to result in gelation. The temperature should not be harmful to the ISG material. Good results are obtained within the temperature range of about 0–20° C. It is not necessary to hold the so-adjusted material for any period of time prior to the next step; however, the material may be held, if desired, without detrimental effects.

The protein solution at the appropriate pH (preferably 3.8–4.2) may be diafiltered with at least 4 volume exchanges of water to reduce the alcohol concentration from approximately 17% (Filtrate III) to about 2% alcohol. The efficacy of solvent/detergent as a viral inactivation method is much better at or above ambient temperatures; however, high concentrations of alcohol at these temperatures will denature the IgG molecules. Thus, this inactivation must be performed in low alcohol concentration.

Next, the protein concentration of the so-treated material is adjusted to the level desired for incubation with TNBP/detergent, generally less than 10% protein for maximum viral inactivation. This adjustment is accomplished by conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Prior to addition of TNBP/detergent, the pH may be adjusted within a wide range, depending on the detergent to be used. With Tween 80, the pH may be as low as 3.5, where the IgG starts becoming unstable. With cholate, the pH is adjusted to within the range of 5.0–6.4, preferably about 5.6, prior to addition of TNBP/detergent. Satisfactory cholate solubility during incubation was achieved by adjusting the immunoglobulin solutions to a pH of 5.5 or higher prior to addition of TNBP and sodium cholate. Adjusting the IgG solution to pH values lower than 5.5 is not suitable because the solubility of sodium cholate is highly dependent on pH (cholic acid pK=6.4), with poor solubility at pH 5.5 or lower. Furthermore, maximum viral inactivation during incubation with TNBP/cholate was observed at pH values less than 6.0 in experiments which employed model viruses spiked into IgG solutions. The inactivation of HIV-1 and BVDV (bovine viral diarrhea virus, which is employed as a model for hepatitis C) was accelerated at pH 5.8, with inactivation to the detection limit occurring in 1–2 hours, whereas inactivation to the detection limit required a minimum of 6 hours when pH 7 conditions were used.

Next, the TNBP/detergent is added to the protein solution (preferably less than 8% [w/w], pH 5.8) mixed thoroughly, and then incubated above ambient temperatures, for example 30° C., with continuous agitation or mixing. Target TNBP/cholate levels for optimal viral inactivation during the incubation step should be >3 mg/mL TNBP and >2 mg/mL cholate as defined by Edwards et al. (8) Moreover, for effective viral inactivation, it is important that the solution is essentially free of particulates in order to facilitate thorough mixing of solvent/detergent and IgG solution. After incubation with TNBP/cholate under these conditions, greater than 5.2 $\log_{10}$ reduction of HIV-1 and greater than 4.0 $\log_{10}$ reduction of BVDV were detected.

After completing the incubation which provides the viral inactivation, the solvent and detergent molecules must be removed in order to achieve a final product with low levels of residual TNBP and cholate which would be suitable for intravenous administration. Generally, procedures to remove detergent are also effective in removing TNBP, and vice versa. Very low levels of TNBP and cholate in the final container can be achieved by a combination of filtration, diafiltration and hydrophobic chromatography. After completing the incubation, the majority of cholate (and TNBP) can be removed from the protein solution by filtration, providing the solution had been previously adjusted to a lower pH value such as 4.0, because sodium cholate is sparingly soluble in aqueous solutions at such pH values. Moreover, all processing steps which follow the solvent/detergent incubation are performed at lower pH values (i.e., 4.0) because IgG molecules are more stable at pH values between 3.5–5.0, in low ionic strength solutions. (2) Thus, after incubation with TNBP/cholate, the protein solution is adjusted to approximately pH 4.0 and incubated at 0–8° C. in order to promote cholate precipitation. Next, filtration is employed to remove the precipitated cholate from the IgG solution.

The so-treated solution is diafiltered with at least four volume exchanges of water to reduce the ionic strength and

to remove additional TNBP and cholate. After or during the above treatment, the pH is measured and maintained within the range of about 3.5–5.0. The protein concentration of the so-treated material is adjusted to 10–30%, usually 13% (w/v) by employing conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Again the pH of the preparation is maintained within the range of about 3.5–5.0, preferably about 3.8–4.2.

In the present invention, hydrophobic chromatography is employed to remove the TNBP and cholate not eliminated by the filtration and diafiltration steps, and thus provide a final product with low levels of residual TNBP and cholate which is suitable for intravenous administration. Hydrophobic chromatography is a method for TNBP removal from protein solutions that has fewer drawbacks and limitations than other available methods such as oil extraction, ion exchange or affinity chromatography. In part, this is because the protein of interest (IgG) remains in solution throughout the TNBP removal process. Polystyrene-based resins (typically PLRP-S from Polymer Laboratories, Amherst, Mass.) were used to remove the solvent/detergent from solution, as we have found the polystyrene-based resins to be superior to other resins, such as silica-based C-18 resins.

Next, the ISG preparation is adjusted to 5% or 10% protein, and treated to render it tonic, i.e., to render it compatible with physiological conditions, or render it physiologically acceptable upon injection. In a preferred embodiment, the tonicity is adjusted to about 230 to about 490 mosmol/kg solvent. More preferably, the tonicity range is from about 250 to about 350 mosmol/kg solvent, and most preferably the tonicity range is from about 260 to about 325 mosmol/kg solvent. The 5% formulation (5% IGIV) is made tonic by the addition of 10% maltose. The 10% formulation contains 0.2 M glycine in order to achieve an isotonic preparation without large quantities of sugar. The product with either formulation (Gamimune®N 5% or Gamimune®N 10%) experiences shifts in molecular distribution (antibody aggregation) when the ionic strength of the low pH solution is increased. Therefore, sodium chloride, which is often used to achieve tonicity, should not be used.

The so-treated solution is incubated at pH 4.25 under low ionic strength conditions (NLT 21 days at 20–27° C. preferred) in order to provide a lowering of ACA levels. The ionic strength is determined according to Perrin (18), and in a preferred embodiment the ionic strength should be less than about 0.001M. Elevated ACA levels were always detected at this stage of all TNBP/cholate treated IGIV preparations (regardless of process scale); however, ACA levels are gradually lowered by incubation at pH 4.25 under low ionic strength conditions (Tables 3, 5–7). While there is no strict rule for determining when the ACA level is low enough to be an acceptable level suitable for intravenous administration, IGIV preparations should have ACA levels as low as possible.

The Figure depicts the typical average reduction of ACA observed in 5% IGIV solutions following SD treatment. For a 5% ISG formulation the acceptable level suitable for intravenous administration preferably would be less than about 45 CH_{50} units/mL, and more preferably less than about 30 CH_{50} units/mL. For a 10% ISG formulation, the acceptable level suitable for intravenous administration preferably would be less than about 60 CH_{50} units/mL, and more preferably less than about 45 CH_{50} units/mL. As used herein, one unit of ACA activity (one CH_{50} unit) is defined as the amount of protein capable of activating 50% of the complement in an optimally titered complement and red blood cell/hemolysin system. The assay measures the amount of complement that is bound by the mixture of standardized amounts of complement and protein. See refs. 19–20 for a discussion of the assay. Briefly, red blood cells that have been sensitized by preincubation with red blood cell antibodies are added to the complement/protein mixture. In the presence of free complement (not already bound by the protein) these sensitized cells will lyse, releasing hemoglobin which can be quantitated as a measure of the degree of lysis. In parallel, sensitized red blood cells are also added to a buffer control-complement mixture, whose degree of lysis is defined as 100%. The difference between the actual amount of complement needed to give 100% lysis and the amount of complement remaining unbound in the presence of protein equals the amount of complement actually bound by the protein, or anticomplement activity.

Results

Anticomplement Activity of ISG Resulting From Viral Inactivation Process

To establish the effect of the SD viral inactivation process on solutions containing ISG which are formulated according to the Tenold '608 patent, the experiments depicted in Table 1 were performed. The starting material (SM) was Cohn process filtrate III which had been ultrafiltered to about 5% protein and then diafiltered with four volumes of water.

In the control experiment, incubation (–)/SD (–), the SM was not subjected to any incubation or solvent/detergent treatment. In the incubation (+)/SD (–) experiment, the pH of the SM was adjusted to 7.0, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & Tween 80 (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL Tween 80 were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & cholate (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL cholate were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. The solutions in each experiment were then diafiltered with four volumes CWFI (cold water for injection) and concentrated by ultrafiltration. After addition of dry maltose to 10% w/v, the 5% IGIV solution (pH 4.25) was filtered through a 0.2 μm filter.

TABLE 1

Anticomplement activity in 5% IGIV produced by variations of the Solvent/Detergent IGIV Process

	ACA (CH_{50}/mL)
Control (no solvent/detergent treatment, no 30° C. incubation)	25
Incubate at 30° C. for 10 hr (no solvent/detergent)	22
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL Tween 80	68
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP NLT 2 mg/mL cholate	>100

*These samples were assayed for ACA after final compounding according to the Tenold '608 patent, but they were not incubated at pH 4.25 and 22° C. prior to analysis.

The results listed in Table 1 show that levels of ACA increased in IgG samples after incubation with TNBP/cholate or TNBP/Tween 80. ACA levels were not elevated in IgG samples that were incubated for 10 hr at 30° C. in the

absence of solvent/detergent. These results suggest that ACA levels of IGIV samples were not elevated by either processing manipulations or incubation for 10 hr at 30° C. in the absence of solvent/detergent.

TABLE 2

Anticomplement activity in 5% IGIV spiked with TNBP/Na cholate

	ACA (CH_{50}/mL)
5% IGIV, no TNBP/cholate	12
5% IGIV with 100 μg/mL · TNBP, 100 μg/mL Na cholate	13

Furthermore, spiking experiments (with TNBP and Na cholate, Table 2) have demonstrated that the elevated anticomplement activity levels were not artifacts caused by carrying out the anticomplement assay in the presence of trace levels of TNBP/Na cholate. Thus, using the prior art SD process for viral inactivation of a solution containing ISG, subsequently formulated according to the Tenold '608 patent, yields a product which has high ACA and is unsuitable for intravenous administration. In a similar experiment, SD treated samples which were not incubated (Table 3, Initial Testing) had ACA levels greater than 100 units.

TABLE 3

Reduction in Anticomplement activity of samples previously treated with TNBP/cholate

Sample	ACA (CH_{50}/mL) Initial Testing (no incubation)	ACA (CH_{50}/mL) After incubation 6 wk. @ 5° C. 3 wk. @ 22° C.
RB21872-16	>100	33
RB21872-17	>100	34
RB21872-18	>100	36
RB21872-20	>100	27

However, when duplicate SD treated samples were incubated for extended periods of time (6 weeks at 5° C. and 3 weeks at 22° C.), the level of ACA was markedly reduced (Table 3, after incubation). This led to further investigation of this surprising observation.

Aggregate Content of ISG Exposed to TNBP/cholate

The samples of the previous experiment (Table 3, Initial Testing) were analyzed by size exclusion (gel permeation) HPLC immediately after compounding to determine the extent of aggregation of the IGIV at the initial time point. HPLC analysis shows nearly complete monomer content in the samples. (Table 4).

TABLE 4

HPLC analysis of non-incubated 5% IGIV samples (Table 3 Initial)

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
RB21872-16, initial	0.140	0.00	99.86	0.00
RB21872-17, initial	0.146	0.00	99.85	0.00
RB21872-18, initial	0.124	0.00	99.88	0.00
RB21872-20, initial	0.172	0.00	99.83	0.00

Previously, high IgG aggregate levels were shown to correlate with high anticomplement activity. However, results from analysis of the samples show the level of ACA in the samples to be greater than 100 units. (Table 3, 'Initial Testing') The HPLC analysis shows that the high ACA following the TNBP/cholate treatment was not due to the presence of aggregated IgG molecules.

Varied Conditions of Time and Temperature

The SM was the same as in the previous experiment, and experimental conditions were similar with the following changes. The solutions were treated with TNBP/cholate at pH 7.0 and then were compounded to 5% IGIV, 10% maltose, pH 4.25, as above. The ACA was assayed immediately after final compounding, after a first incubation for nine days at 5° C., and after a second incubation for 21 days at either 22° C. or 5° C. The results are presented in Table 5.

TABLE 5

ACA of TNBP/cholate treated IGIV samples

Sample Point	ACA (CH_{50}/mL)
Intermediate Samples	
Initial sterile bulk	>100
Incubated 9 d. @ 5° C.	>100
Final Incubation	
21 d. @ 22° C.	49
21 d. @ 5° C.	71

In the initial sterile bulk sample, which was treated with TNBP/cholate at pH 7.0, the level of ACA was again greater than 100 units for the initial time point, confirming the observations noted in Table 3. Upon incubation at 5° C. for nine days, the ACA remained greater than 100 units. The final incubation step at either 5° C. or 22° C. shows that the reduction in ACA is dependent on temperature, with faster reduction in ACA observed at higher temperatures.

Effect of pH During Solvent/detergent Treatment on ACA

ACA levels were evaluated after incubation with TNBP/cholate at pH 5.8 because better viricidal activity was observed at pH values less than 6.0. Generally, the non-incubated sterile bulk samples of material incubated at pH 5.8 had lower ACA levels than the pH 7.0 samples, but the trend of lowering ACA upon incubation was repeated in the pH 5.8 samples. In fact, the ACA levels continue to decrease beyond the 21 day incubation in samples that initially had elevated ACA levels after incubation with TNBP/cholate at pH 5.8 (Table 6). As was previously noted for the samples incubated at pH 7.0, the lowering of ACA was not due to decreasing levels of aggregated IgG molecules because the material treated at pH 5.8 was essentially monomeric IgG prior to 22° C. incubation (HPLC analysis, sample A4, Table 8).

TABLE 6

Sample A4 - ACA upon extended incubation

Incubation at 22° C. (days)	CH_{50}/mL
0	122
10	73
19	55
25	56
28	45
30	40
34	39
41	33

TABLE 6-continued

Sample A4 - ACA upon extended incubation

Incubation at 22° C. (days)	CH_{50}/mL
48	30
55	29

Similar results were achieved with samples formulated to 10% IGIV, 0.2 M glycine in the sterile bulk stage. Upon incubation at low ionic strength at pH 4.25 for 10 and 21 days, the levels of ACA were seen to decline in both 5% IGIV samples and 10% IGIV samples. (Table 7) The decrease in ACA can thus be observed over a range of ISG concentrations and over a range of pH values for the solvent/detergent treatment. (Tables 3, 5, 7) HPLC analysis (Table 8) of the sterile bulk samples presented in Table 7 confirmed that the elevated ACA levels were not due to aggregation of ISG molecules.

TABLE 7

ACA of samples treated with TNBP/cholate at pH 5.8

Sample	Sterile bulk (day zero) (CH_{50}/mL)	10 days incubation at 20–27° C. (CH_{50}/mL.)	21 days incubation at 20–27° C. (CH_{50}/mL.)
A1 (5% IGIV)	43	ND	10
A2 (5% IGIV)	31	14	15
A3 (5% IGIV)	44	15	12
A4 (5% IGIV)	122	73	55
B1 (10% IGIV)	>100	48	46
B2 (10% IGIV)	49	36	30
B3 (10% IGIV)	53	ND	37

Taken together, the above results suggest that ISG products which have been subjected to a solvent/detergent viral inactivation process resulting in an undesirable ACA increase can be made suitable for IV administration by incorporating an additional incubation step under the conditions described here to reduce the ACA to an acceptable level.

TABLE 8

HPLC Analysis of sterile bulk samples treated with TNBP/cholate at pH 5.8

Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
A2	0.140	0.00	99.86	0.00
A3	0.146	0.00	99.85	0.00
A4	0.124	0.00	99.88	0.00

CONCLUSION

The ACA increase resulting from the solvent/detergent treatment of the IGIV (antibody) solution appears to be an unavoidable secondary effect of TNBP/detergent treatment to inactivate viruses in the solution. I have discovered that by incubating the solution of IGIV at low pH (4.25) and low ionic strength (0.001M) for a relatively long period of time (at least about 10 days), the ACA gradually decreases over the period of incubation.

The prior art discloses a method of producing IGIV (the Tenold '608 patent) using low pH and low ionic strength. The Tenold '608 method omits the viral inactivation step, and thus avoids the problem of increased ACA, but the possibility of viral activity remains. Unlike Tenold, incubation is an essential aspect of the present invention for reducing the ACA.

The Neurath et al. '573 patent teaches the solvent/detergent viral inactivation step. However, Neurath '573 does not mention controlling the pH and also does not mention any consequences of the process relating to ACA. Elevated ACA levels were detected at the sterile bulk stage of TNBP/cholate treated IGIV preparations. However, ACA levels decreased upon incubation for at least about 10 days at pH 4.25, low ionic strength, and not less than about 20° C. (See Tables 5–7) The prior art describes several approaches to lowering ACA levels of purified IgG preparations, including removal of IgG aggregates. (11) IgG aggregates have been shown to activate the complement system in vivo. (1) In the present invention, however, lowering of IgG ACA was not due to decreasing levels of IgG aggregates because these TNBP/cholate treated IGIV preparations contained low levels of aggregated IgG (as measured by HPLC, Tables 4, 8) prior to incubation under such conditions.

It would be desirable to produce substantially virus-free IGIV, but following the prior art results in a product with an unacceptable level of ACA. Note that Tenold '608 states that the product is substantially free of ACA, but use of the SD process in conjunction with Tenold '608 does result in high levels of ACA: experimental results reported here show that treating ISG solutions with the SD process and then formulation according to the Tenold '608 patent leads to a product with high ACA. (See Tables 1, 3, 5–7) The surprising finding reported here is that a follow-up (terminal) incubation step lowers the ACA of the solvent/detergent treated solution. The typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation are compared in the Figure. The present invention thus includes a previously unobserved method of reducing the ACA by incubating under controlled conditions of pH, temperature, and ionic strength for a period of time, thus allowing the product to be administered by intravenous injection.

Mitra '714 does not suggest the use of a S/D process but, instead, reports that a relatively brief incubation of an ISG product under similar conditions results in a substantially virus free preparation. (10) However, employing incubation under such conditions to provide a lowering of anticomplement activity is a novel application of these incubation conditions which were previously employed in the IGIV process for inactivation of enveloped viruses.

The newly developed IGIV process reported here, which includes an additional internationally accepted viral inactivation procedure (treatment with TNBP/cholate), generates IgG preparations which have low ACA levels and are suitable for IV administration. The major advantage is that an IGIV product with improved safety can be obtained by a two-step process that includes a TNBP/cholate treatment for viral inactivation and incubation under conditions that afford low ACA levels that are suitable for IV administration.

The above disclosure is intended to illustrate the invention, and it is thought variations will occur to those skilled in the art. Accordingly, it is intended that the scope of the invention should be limited only by the claims below.

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What is claimed is:

1. A method of treating a solution of antibodies which may have virus activity, the method comprising

a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and

b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

2. The method of claim 1, wherein the anticomplement activity is reduced to less than about 60 CH_{50} units/mL.

3. The method of claim 1, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

4. The method of claim 3, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 30 CH_{50} units/mL.

5. The method of claim 1, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 60 CH_{50} units/mL.

6. The method of claim 5, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 45 CH_{50} units/mL.

7. The method of claim 1, wherein the incubation is for at least about ten days.

8. The method of claim 1, wherein the pH is maintained within a range of about 3.5 to about 5.0.

9. The method of claim 1, wherein the temperature is maintained within a range of 2° C. to 50° C.

10. The method of claim 1, wherein the ionic strength is less than about 0.001 M.

11. The method of claim 1, wherein at least about 99% of the antibodies are monomeric.

12. The method of claim 1, comprising the further step of adjusting the tonicity of the solution to a physiologic value under such conditions that the ionic strength is not appreciably altered.

13. The method of claim 12, wherein the tonicity of the solution is adjusted by adding a carbohydrate to the solution.

14. The method of claim 13, wherein the carbohydrate used is maltose.

15. The method of claim 12, wherein the tonicity of the solution is adjusted to a range of about 230 to about 490 mosmol/kg solvent.

16. The method of claim 15, wherein the tonicity of the solution is adjusted to a range of about 274 to about 309 mosmol/kg solvent.

17. The method of claim 12, wherein the tonicity of the solution is adjusted by adding an amino acid to the solution.

18. The method of claim 17, wherein the amino acid used is glycine.

19. The method of claim 1, wherein the trialkylphosphate is tri-n-butyl phosphate and the detergent is selected from polysorbate 80 and sodium cholate.

20. The method of claim 1, wherein the solution has a pH between about 3.5 and about 6.0 during step a).

21. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001 M, a pH between about 3.5 and about 5.0, an antibody concentration of about 5% wt./wt., and a maltose concentration of about 10% wt./wt.

22. The preparation of claim 21, wherein the pH is about 4.25.

23. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.

24. The preparation of claim 23, wherein the pH is about 4.25.

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